

REMARKS

Applicant respectfully requests reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow.

Applicants have amended pages 11 and 12 of the specification to overcome the objections raised by the Examiner.

Claims 1-15 were pending in the application. Claims 4-15, drawn to a non-elected invention, remain withdrawn from further consideration. Applicants reserve their right to file one or more divisional applications directed to any non-elected subject matter.

Applicants have revised claims 1-3 and added new claims 16-17. Upon entry of these amendments, claims 1-3 and 16-17 will be pending.

A detailed listing is presented, with an appropriate defined status identifier, of all claims that are or were in the application, irrespective of whether the claim(s) remain under examination. As noted, these amendments do not go beyond the original specification.

Objection to the Specification

The Examiner has noted that the terms "MARATHON-READY" and "PROMEGA" are trademarks and should be capitalized and accompanied with a generic terminology. With respect to the term "MARATHON-READY," Applicants have amended the specification by deleting the sentence at page 11, lines 7-8 and introducing a new sentence that reads as follows:

The 5'-RACE method was conducted following the protocol provided in the "Marathon-Ready™ cDNA Kit (mouse testis), which is commercially available from Clontech.

In addition, Applicants wish to point out that "PROMEGA" is a name of a manufacturer and, therefore, is not a tradename and does not have a generic terminology.

Furthermore, Applicants have assigned "SEQ ID NO:23" to the sequence on page 12, lines 17-18. Support for this amendment is found on page 13-14 of the enclosed Substitute Sequence Listing filed June 4, 2001 (Appendix A).

Rejection Under 35 U.S.C. § 112, Second Paragraph

The examiner alleges that the phrase “functionally equivalent” in claims 2 and 3, is indefinite and unclear because this phrase, as defined in the specification, is not indicative of the specific biological activities of the protein.

Contrary to the Examiner’s allegation, the specification defines this phrase as a mutant protein having a “biochemical and/or biological activity” equivalent to the natural protein” and provides two examples of specific biological activities, namely, metal-binding activity and testicular cell differentiation-inducing activity (see specification at page 5, lines 7-9). However, to expedite prosecution, claims 2 and 3 have been amended to recite an isolated protein having testicular cell differentiation activity.

In light of the above remarks and foregoing amendment, Applicants submit that this rejection is moot and should be withdrawn.

Rejection Under 35 U.S.C. § 101

The examiner rejects claims 1-3 because these claims are drawn to non-statutory subject matter. To obviate this rejection, Applicants have followed the Examiner’s suggestion by amending claims 1-3 to recite “an isolated protein...” Thus, this amendment renders the rejection moot.

Rejections Under 35 U.S.C. § 102

Finally, the examiner alleges that claims 2 and 3 lack novelty over Zon *et al.* because Zon teaches a DNA that encodes a mouse Tbc1 polypeptide “which likes tesmin, plays a role in testicular regulation.” Moreover, the Examiner contends that Zon’s Tbc1 polypeptide is “functionally equivalent to SEQ ID NO:4.” In addition, the Examiner alleges that (i) “by sufficient addition, deletion and/or addition of SEQ ID NO:4..., one could obtain the protein of Zon *et al.*”; and (2) the sequence of Zon *et al.* “would hybridize under some conditions to the DNA that encodes SEQ ID NO:4.” Applicants traverse the rejection.

In response, Applicants have amended claim 2 by deleting the phrase “have been replaced, deleted, and/or added, and which is functionally equivalent to the protein of claim 1” and by inserting the phrase “wherein said protein has testicular

found throughout the entire specification, particularly on page 5, lines 7-9.

To distinguish the protein of the cited art having similar biological activity, Applicants further revise claims 2 and 3 to define the claimed protein in terms of its homology or stringency of hybridization conditions. The description on page 6, lines 12-14 of the specification, serves as support for the specific homology. The stringent conditions for hybridization are specifically exemplified on page 6, lines 17-25 of the specification.

Applicants have introduced new claims 16-17 to recite an isolated protein having a higher homology. Support for this amendment is found in the specification on page 6, lines 12-16.

To further distinguish the claimed protein over the protein of Zon *et al.*, Applicants submit a BLAST alignment search result that compares the sequences of these two protein. As shown in Appendix B, there is no significant homology/similarity between the amino sequences of these two proteins or their corresponding nucleotide sequences that encode them. In fact, the length of both amino acid sequences or nucleotide sequences are also different. Thus, the claimed protein, Tesmin, is distinct from the mouse Tbc1 of Zon *et al.*

Based on the foregoing amendments and above remarks, Applicants believe that these rejections are rendered moot and should be withdrawn.

CONCLUSION

In view of the foregoing amendments and remarks, favorable reconsideration and allowance of this application are requested. An early notice in this regard is earnestly solicited. In the event that any issues remain, the Examiner is invited to contact the undersigned with any proposal to expedite prosecution.

Respectfully submitted,

Date Dec, 16, 2003

FOLEY & LARDNER
Customer Number: 22428



22428

PATENT TRADEMARK OFFICE

Telephone: (202) 672-5569

Facsimile: (202) 672-5399

Stephen B. Maebius
Attorney for Applicants
Registration No. 35,264

Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 19-0741 for any such fees; and applicant(s) hereby petition for any needed extension of time.



APPENDIX A

Docket No. 084335/0127

Receipt is hereby acknowledged of the following:

Applicants: Takashi Sugihara et al.
Serial No.: 09/743,237
Filing Date: January 5, 2001

For: TESTIS-SPECIFIC DIFFERENTIATION-REGULATORY FACTOR

1. Check No. 12418 for \$240
2. Transmittal Letter to the U.S. Designated/Elected Office (2nd Submission) including; Copies of Form PCT/DO/EO/905 & 920; Statement to Support Filing and Submission in Accordance with 37 C.F.R. §§ 1.821-1.825; Substitute Sequence Listing (15 pages); and Associate Power of Attorney; Information Disclosure Statement and Form PTO-1449 with 1 attachment; Declaration and Power of Attorney; disk containing sequence listing; and Petition for Extension of Time for 1 month

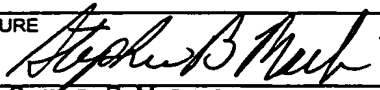
Date Due: June 30, 2001
Date: June 4, 2001

Return to: SBM/mkm
Inspected by: *BM*





FORM PTO-1390 (Modified) (REV 5-93)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				084335/0127	
				U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5) 09/743,237	
INTERNATIONAL APPLICATION NO. PCT/JP99/03859		INTERNATIONAL FILING DATE July 16, 1999		PRIORITY DATE CLAIMED July 17, 1998	
TITLE OF INVENTION TESTIS-SPECIFIC DIFFERENTIATION-REGULATORY FACTOR					
APPLICANT(S) FOR DO/EO/US Takashi SUGIHARA, Renu WADHWA, Sunil C. KAUL, Youji MITSUI					
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:					
1. <input type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.					
2. <input checked="" type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.					
3. <input type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).					
4. <input type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19 th month from the earliest claimed priority date.					
5. <input type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). <input type="checkbox"/> has been transmitted by the International Bureau. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US)					
6. <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).					
7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). <input type="checkbox"/> have been transmitted by the International Bureau. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. <input type="checkbox"/> have not been made and will not be made.					
8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).					
9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).					
10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).					
Items 11. to 16. below concern other document(s) or information included:					
11. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.					
12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.					
13. <input type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.					
14. <input type="checkbox"/> A substitute specification.					
15. <input type="checkbox"/> A change of power of attorney and/or address letter.					
16. <input checked="" type="checkbox"/> Other items or information: Copies of Form PCT/DO/EO/905 & 920; Statement to Support Filing and Submission in Accordance with 37 C.F.R. §§ 1.821-1.825; Substitute Sequence Listing (15 pages); Associate Power of Attorney; and Petition for Extension of Time for 1 month					

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.50) 09/743,237		INTERNATIONAL APPLICATION NO. PCT/JP99/03859		ATTORNEY'S DOCKET NUMBER 084335/0127	
17. <input checked="" type="checkbox"/> The following fees are submitted:				CALCULATIONS	
Basic National Fee (37 CFR 1.492(a)(1)-(5): Search Report has been prepared by the EPO or JPO.....\$860.00					
International preliminary examination fee paid to USPTO (37 CFR 1.482).....\$690.00					
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))\$710.00					
Neither international preliminary examination fee (37 CFR 1.482) nor International search fee (37 CFR 1.445(a)(2)) paid to USPTO \$1,000.00					
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)\$100.00					
ENTER APPROPRIATE BASIC FEE AMOUNT =					
Surcharge of \$130.00 for furnishing the oath or declaration later than 20 Months from the earliest claimed priority date (37 CFR 1.492(e))					
Claims	Number Filed		Included in Basic Fee	Extra Claims	Rate
Total Claims	15	-	20	= 0	x \$18.00
Independent Claims	2	-	3	= 0	x \$80.00
Multiple dependent claim(s) (if applicable)				\$270.00	
TOTAL OF ABOVE CALCULATIONS =				\$	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).					
SUBTOTAL =					
Processing fee of \$130.00 for furnishing English translation later the 20 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$130.00	
TOTAL NATIONAL FEE =					
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +					
TOTAL FEES ENCLOSED =				\$130.00	
				Amount to be: refunded \$	
				charged \$	
a. <input checked="" type="checkbox"/> A check in the amount of \$130.00 to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. <u>19-0741</u> in the amount of \$0.00 to the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>19-0741</u> . A duplicate copy of this sheet is enclosed.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO:					
Foley & Lardner 3000 K Street, N.W., Suite 500 Washington, D.C. 20007-5109			SIGNATURE  NAME STEPHEN B. MAEBIUS		
REGISTRATION NUMBER 35,264					

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty. Docket No: 084335/0127

In re patent application of

SUGIHARA, TAKASHI et al.

Serial No. 09/743,237

Filed: January 5, 2001

For: TESTIS-SPECIFIC DIFFERENTIATION-REGULATORY FACTOR



STATEMENT TO SUPPORT FILING AND SUBMISSION IN
ACCORDANCE WITH 37 C.F.R. §§ 1.821-1.825

Assistant Commissioner for Patents
Washington, D.C. 20231
Box SEQUENCE

Sir:

In connection with a Sequence Listing submitted concurrently herewith, the undersigned hereby states that:

1. the submission, filed herewith in accordance with 37 C.F.R. § 1.821(g), does not include new matter;

2. the content of the attached paper copy and the attached computer readable copy of the Sequence Listing, submitted in accordance with 37 C.F.R. § 1.821(c) and (e), respectively, are the same; and

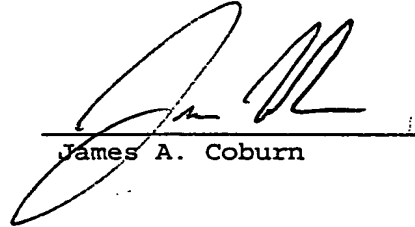
3. all statements made herein of their own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United

Serial No. 09/743,237

States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

Respectfully submitted,

Aug 31, 2001
Date


James A. Coburn

HARBOR CONSULTING
Intellectual Property Services
1500A Lafayette Road
Suite 262
Portsmouth, N.H.
800-318-3021



1

SEQUENCE LISTING

<110> SUGIHARA, TAKASHI
WADHWA, RENU
KAUL, SUNIL C.
MITSUI, YOUJI

<120> TESTIS-SPECIFIC DIFFERENTIATION-REGULATORY FACTOR

<130> 084335/0127

<140> 09/743,237

<141> 2001-01-05

<150> PCT/JP99/03859

<151> 1999-07-16

<160> 24

<170> PatentIn Ver. 2.1

<210> 1

<211> 2241

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (651)..(1535)

<400> 1

```
tatcctgtgg gttggccccg ggcagcaggc tctcagcagg ctcaggcacc acaaggata 60
cacagtgtgt gttcctggcc tgttggactt gtgactccac ccacctccgc cccagcaggg 120
ctagggatag aaccagggc cttttgcgtg ttctgcagat agtccttcage ctggtagttt 180
ggggttggct gggagatttt tttttcttca caccaaagac ttccattatt gaggattttt 240
tcagttgatg atctcccccc tctgtaagat aaggacagc tctttaaac tatgtagagt 300
tttgatgaat tctgcttctc aaccatattg ctaagctata tagcaattcc ttgaaattgc 360
tatataactt aggagaacct ctgattctcc tgcctctaca tctgagtgc taggtgtaca 420
gggggaaatc attttggtga gactccgatg aactactgcc aggttcccaa ggcagcaagc 480
aagcaagaaa aagtgttgaa atcaaagaag caggtggttag tgtgccaggc ggcagccctg 540
aagacgcagc tttccaggcc cctctggctc aggaatcctg ttgcaagttc ccatcatccc 600
aggaggcaga ggaggcctcc agctgccctc ggaagaaaga ctccagcccc atg gtg      656
                                     Met Val
                                     1
att tgt cag ctg aaa gga ggc gcc cag atg ctc tgc ata gac aac tgt      704
Ile Cys Gln Leu Lys Gly Gly Ala Gln Met Leu Cys Ile Asp Asn Cys
      5              10              15
```

ggc gcg agg gag ctc aaa gcg ctc cat ctg ctt cct cag tac gat gac	752
Gly Ala Arg Glu Leu Lys Ala Leu His Leu Leu Pro Gln Tyr Asp Asp	
20 25 30	
cag agc agt ttc cct cag tca gag ctc cct aag cca atg aca act tta	800
Gln Ser Ser Phe Pro Gln Ser Glu Leu Pro Lys Pro Met Thr Thr Leu	
35 40 45 50	
gtg gga aga ctt ctg cca gta cca gcg aag tta aat ctc atc aca cag	848
Val Gly Arg Leu Leu Pro Val Pro Ala Lys Leu Asn Leu Ile Thr Gln	
55 60 65	
gtt gat aat gga gct ctc cca tca gct gtc aat ggg gct gcc ttt ccc	896
Val Asp Asn Gly Ala Leu Pro Ser Ala Val Asn Gly Ala Ala Phe Pro	
70 75 80	
tct gga cct gct ctg caa ggg cca ccc aaa ata act ctg tct ggg tac	944
Ser Gly Pro Ala Leu Gln Gly Pro Pro Lys Ile Thr Leu Ser Gly Tyr	
85 90 95	
tgt gac tgc ttc tcc agc ggg gac ttc tgc aac agc tgc agc tgc aac	992
Cys Asp Cys Phe Ser Ser Gly Asp Phe Cys Asn Ser Cys Ser Cys Asn	
100 105 110	
aac ctg cgc cat gag ctc gag cgc ttc aaa gcc ata aag gcg tgt ctt	1040
Asn Leu Arg His Glu Leu Glu Arg Phe Lys Ala Ile Lys Ala Cys Leu	
115 120 125 130	
gat aga aat cct gaa gct ttc caa cca aaa atg ggg aaa ggc cgt ctg	1088
Asp Arg Asn Pro Glu Ala Phe Gln Pro Lys Met Gly Lys Gly Arg Leu	
135 140 145	
gga gct gct aaa ctt cga cac agc aaa ggg tgc aac tgt aag cgc tca	1136
Gly Ala Ala Lys Leu Arg His Ser Lys Gly Cys Asn Cys Lys Arg Ser	
150 155 160	
ggc tgc ctg aag aac tac tgt gag tgc tat gag gcc aaa atc atg tgt	1184
Gly Cys Leu Lys Asn Tyr Cys Glu Cys Tyr Glu Ala Lys Ile Met Cys	
165 170 175	
tct tcc att tgc aaa tgc att gct tgc aaa aac tat gaa gaa agt cca	1232
Ser Ser Ile Cys Lys Cys Ile Ala Cys Lys Asn Tyr Glu Glu Ser Pro	
180 185 190	
gaa cga aaa atg ctg atg agc aca ccc cac tac atg gag cct ggg gac	1280
Glu Arg Lys Met Leu Met Ser Thr Pro His Tyr Met Glu Pro Gly Asp	
195 200 205 210	
ttt gag agc agc cat tat ttg tcc cca gcc aag ttc tca gga cct cca	1328
Phe Glu Ser Ser His Tyr Leu Ser Pro Ala Lys Phe Ser Gly Pro Pro	
215 220 225	
aaa ctg aga aaa aat agg cag gcc ttc tcc tgt atc tcc tgg gaa gta	1376
Lys Leu Arg Lys Asn Arg Gln Ala Phe Ser Cys Ile Ser Trp Glu Val	
230 235 240	

gtg gag gcc aca tgt gcc tgc ctg ctg gcc cag ggt gag gaa gca gag 1424
 Val Glu Ala Thr Cys Ala Cys Leu Leu Ala Gln Gly Glu Glu Ala Glu
 245 250 255

cag gag cac tgt tcc cca agc ttg gct gag cag atg atc ctg gag gag 1472
 Gln Glu His Cys Ser Pro Ser Leu Ala Glu Gln Met Ile Leu Glu Glu
 260 265 270

ttt gga agg tgc ctg tgc cag att ctc cac atc gag ttc aag tcc aag 1520
 Phe Gly Arg Cys Leu Ser Gln Ile Leu His Ile Glu Phe Lys Ser Lys
 275 280 285 290

ggg ctg aaa att gag tagcgtgcaa gctggtaaag gggaatgcct gtggcaagcc 1575
 Gly Leu Lys Ile Glu
 295

tcagccctgg gaatctgcac cgaggaagct ggtgcccagg gaggagcaga ggccgcgcat 1635
 catggccagg tcagctgtga ggtctgagt atctgcatgg tactggccag cctactcaag 1695
 gtatcctaaa gtgcaagcag gcagagccac cctggggatg gacactggcc ctctgtccc 1755
 tggggaggcc ctctggggac tccctgccct gcataaaaag agggtgattt tctacttggt 1815
 gttatgtgtt tgctttcaaa ttgcttagta gtacctccat tcaagttatt atgagccagc 1875
 ctcaagttag agagctaggc tcttcttcag gtggactctg cccaaatcac atacaagtca 1935
 ggtggccatc aggggttttt ccaggccagg cctgtgacag gagatatggg aggggggtcg 1995
 ggtagagct gggtttgttt ggattttttg cgtttttttc ttctgtatt tctgcttgaa 2055
 gtgagaaaac ttgtctctg tccaaccttt tctccataat tactgctgca cggtcgctg 2115
 ctgaccagtc acagtgcct cagacaccag aaggtgaggt ggcttattat gccacactt 2175
 tgtgttttgt tgtgagaata aacctttcca gactcccaa aaaaaaaaaa aaaaaaaaaa 2235
 aaaaaa 2241

<210> 2
 <211> 1861
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (271)..(1155)

<400> 2
 ccagacgacg ccgctccgcc tccgcctac agcgtgcacg tggtatcgtc gttacttccc 60
 ggtgctcgcg ggcccgcgct gttgccgcta agcgcaggag tgcgcgtgat ccagttgaa 120
 atcaaagaag caggtggtag tgtgccaggc ggcagccctg aagacgcagc tttccaggcc 180
 cctctggctc aggaatcctg ttgcaagttc ccatcatccc aggaggcaga ggaggcctcc 240

agctgccctc ggaagaaaga ctccagcccc atg gtg att tgt cag ctg aaa gga	294
Met Val Ile Cys Gln Leu Lys Gly	
1 5	
ggc gcc cag atg ctc tgc ata gac aac tgt ggc gcg agg gag ctc aaa	342
Gly Ala Gln Met Leu Cys Ile Asp Asn Cys Gly Ala Arg Glu Leu Lys	
10 15 20	
gcg ctc cat ctg ctt cct cag tac gat gac cag agc agt ttc cct cag	390
Ala Leu His Leu Leu Pro Gln Tyr Asp Asp Gln Ser Ser Phe Pro Gln	
25 30 35 40	
tca gag ctc cct aag cca atg aca act tta gtg gga aga ctt ctg cca	438
Ser Glu Leu Pro Lys Pro Met Thr Thr Leu Val Gly Arg Leu Leu Pro	
45 50 55	
gta cca gcg aag tta aat ctc atc aca cag gtt gat aat gga gct ctc	486
Val Pro Ala Lys Leu Asn Leu Ile Thr Gln Val Asp Asn Gly Ala Leu	
60 65 70	
cca tca gct gtc aat ggg gct gcc ttt ccc tct gga cct gct ctg caa	534
Pro Ser Ala Val Asn Gly Ala Ala Phe Pro Ser Gly Pro Ala Leu Gln	
75 80 85	
ggg cca ccc aaa ata act ctg tct ggg tac tgt gac tgc ttc tcc agc	582
Gly Pro Pro Lys Ile Thr Leu Ser Gly Tyr Cys Asp Cys Phe Ser Ser	
90 95 100	
ggg gac ttc tgc aac agc tgc agc tgc aac aac ctg cgc cat gag ctc	630
Gly Asp Phe Cys Asn Ser Cys Ser Cys Asn Asn Leu Arg His Glu Leu	
105 110 115 120	
gag cgc ttc aaa gcc ata aag gcg tgt ctt gat aga aat cct gaa gct	678
Glu Arg Phe Lys Ala Ile Lys Ala Cys Leu Asp Arg Asn Pro Glu Ala	
125 130 135	
ttc caa cca aaa atg ggg aaa ggc cgt ctg gga gct gct aaa ctt cga	726
Phe Gln Pro Lys Met Gly Lys Gly Arg Leu Gly Ala Ala Lys Leu Arg	
140 145 150	
cac agc aaa ggg tgc aac tgt aag cgc tca ggc tgc ctg aag aac tac	774
His Ser Lys Gly Cys Asn Cys Lys Arg Ser Gly Cys Leu Lys Asn Tyr	
155 160 165	
tgt gag tgc tat gag gcc aaa atc atg tgt tct tcc att tgc aaa tgc	822
Cys Glu Cys Tyr Glu Ala Lys Ile Met Cys Ser Ser Ile Cys Lys Cys	
170 175 180	
att gct tgc aaa aac tat gaa gaa agt cca gaa cga aaa atg ctg atg	870
Ile Ala Cys Lys Asn Tyr Glu Glu Ser Pro Glu Arg Lys Met Leu Met	
185 190 195 200	
agc aca ccc cac tac atg gag cct ggg gac ttt gag agc agc cat tat	918
Ser Thr Pro His Tyr Met Glu Pro Gly Asp Phe Glu Ser Ser His Tyr	
205 210 215	

ttg tcc cca gcc aag ttc tca gga cct cca aaa ctg aga aaa aat agg 966
 Leu Ser Pro Ala Lys Phe Ser Gly Pro Pro Lys Leu Arg Lys Asn Arg
 220 225 230

cag gcc ttc tcc tgt atc tcc tgg gaa gta gtg gag gcc aca tgt gcc 1014
 Gln Ala Phe Ser Cys Ile Ser Trp Glu Val Val Glu Ala Thr Cys Ala
 235 240 245

tgc ctg ctg gcc cag ggt gag gaa gca gag cag gag cac tgt tcc cca 1062
 Cys Leu Leu Ala Gln Gly Glu Glu Ala Glu Gln Glu His Cys Ser Pro
 250 255 260

agc ttg gct gag cag atg atc ctg gag gag ttt gga agg tgc ctg tcg 1110
 Ser Leu Ala Glu Gln Met Ile Leu Glu Glu Phe Gly Arg Cys Leu Ser
 265 270 275 280

cag att ctc cac atc gag ttc aag tcc aag ggg ctg aaa att gag 1155
 Gln Ile Leu His Ile Glu Phe Lys Ser Lys Gly Leu Lys Ile Glu
 285 290 295

tagcgtgcaa gctggtaaag gggaatgcct gtggcaagcc tcagccctgg gaatctgcac 1215
 cgaggaagct ggtgcccagg gaggagcaga ggccgcgcac catggccagg tcagctgtga 1275
 ggtctgagtg atctgcatgg tactggccag cctactcaag gtatcctaaa gtgcaagcag 1335
 gcagagccac cctgggggatg gacactggcc ctctgtccc tggggaggcc ctctggggac 1395
 tcctgcctt gcataaaaag agggtgattt tctacttggt gttatgtggt tgctttcaaa 1455
 ttgcttagta gtacctccat tcaagttatt atgagccagc ctcaagttag agagctaggc 1515
 tcttcttcag gtggactctg cccaaatcac atacaagtca ggtggccatc aggggttttt 1575
 ccaggccagg cctgtgacag gagatatggg aggggggtcg ggtagagct gggtttggtt 1635
 ggattttttg cgtttttttc ttctgtatt tctgcttgaa gtgagaaaac ttgtctcctg 1695
 tccaaccttt tctccataat tactgctgca cggtcgcctg ctgaccagtc acagtgcact 1755
 cagacaccag aaggtgaggt ggcttattat gccacactt tgtgttttgt tgtgagaata 1815
 aacctttcca gactcccaa aaaaaaaaaa aaaaaaaaaa aaaaaa 1861

<210> 3

<211> 2134

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (407) .. (1303)

<400> 3

aattcgggggt caaggcgaag ctgcgggggg gcgacagcga cggcggggag ctccctcgggg 60

agtaccccg gatcccagag ctacgcgcgc tggaggacgt cgcgctcctg caggcccccgc 120

agccgcccgc	ctgcaacgtg	cacttcctgt	cctcgctgct	accgcgcac	cgcagcccgc	180
gggtgttttg	cccctggggc	gcctgggtcc	tgcgaaaggag	cctcccaccc	gggcgtccgc	240
atgatcccag	ttgaaatcaa	ggtaagcagg	tggtactact	acaagtaata	atccggaaga	300
agcaactttg	cagaatcttc	ttgctcagga	atcctgttgc	aagttcccat	gggtcccagga	360
actagaggat	gcctcctgct	gttctcttaa	gaaagattcc	aacc	ca atg gtg ata	415
					Met Val Ile	
					1	
tgc caa ttg aaa ggg ggc aca caa atg cta tgt ata gac aat tct aga	463					
Cys Gln Leu Lys Gly Gly Thr Gln Met Leu Cys Ile Asp Asn Ser Arg						
5 10 15						
aca aga gaa cta aaa gca ctc cat ttg gtt cct cag tat caa gat caa	511					
Thr Arg Glu Leu Lys Ala Leu His Leu Val Pro Gln Tyr Gln Asp Gln						
20 25 30 35						
aat aat tat cta cag tca gat gtc cct aaa cca atg act gct tta gta	559					
Asn Asn Tyr Leu Gln Ser Asp Val Pro Lys Pro Met Thr Ala Leu Val						
40 45 50						
ggg aga ttt ttg cca gca tca aca aaa tta aat ctc att aca caa caa	607					
Gly Arg Phe Leu Pro Ala Ser Thr Lys Leu Asn Leu Ile Thr Gln Gln						
55 60 65						
ctt gag gga gcc tta cca tcg gta gtc aac ggg tct gct ttc ccc tcg	655					
Leu Glu Gly Ala Leu Pro Ser Val Val Asn Gly Ser Ala Phe Pro Ser						
70 75 80						
gga tca act ctt cca gga cca cca aaa ata act ttg gct ggg tac tgt	703					
Gly Ser Thr Leu Pro Gly Pro Pro Lys Ile Thr Leu Ala Gly Tyr Cys						
85 90 95						
gac tgc ttt gcc agt ggg gac ttt tgc aac aac tgc aat tgt aat aat	751					
Asp Cys Phe Ala Ser Gly Asp Phe Cys Asn Asn Cys Asn Cys Asn Asn						
100 105 110 115						
tgt tgc aac aac ttg cat cat gat att gaa cgg ttt aaa gcc att aag	799					
Cys Cys Asn Asn Leu His His Asp Ile Glu Arg Phe Lys Ala Ile Lys						
120 125 130						
gca tgt ctt ggt aga aat cca gaa gct ttc cag cca aaa att ggg aag	847					
Ala Cys Leu Gly Arg Asn Pro Glu Ala Phe Gln Pro Lys Ile Gly Lys						
135 140 145						
ggc caa ttg ggc aat gtc aag ccc cag cac aac aaa ggg tgc aac tgc	895					
Gly Gln Leu Gly Asn Val Lys Pro Gln His Asn Lys Gly Cys Asn Cys						
150 155 160						
agg agg tca ggc tgc ctg aag aat tac tgc gag tgc tat gag gcc caa	943					
Arg Arg Ser Gly Cys Leu Lys Asn Tyr Cys Glu Cys Tyr Glu Ala Gln						
165 170 175						

att atg tgt tct tct att tgc aaa tgc att ggt tgc aaa aat tat gaa 991
 Ile Met Cys Ser Ser Ile Cys Lys Cys Ile Gly Cys Lys Asn Tyr Glu
 180 185 190 195

gaa agc cca gaa cga aag aca cta atg agc atg cca aac tac atg cag 1039
 Glu Ser Pro Glu Arg Lys Thr Leu Met Ser Met Pro Asn Tyr Met Gln
 200 205 210

act gga ggt ttg gaa ggc agc cat tac ctg cca cca acg aaa ttt tca 1087
 Thr Gly Gly Leu Glu Gly Ser His Tyr Leu Pro Pro Thr Lys Phe Ser
 215 220 225

gga ctt cca aga ttc agt cac gat agg cgg cct tcc tca tgc atc tcc 1135
 Gly Leu Pro Arg Phe Ser His Asp Arg Arg Pro Ser Ser Cys Ile Ser
 230 235 240

tgg gag gtg gtg gag gcc aca tgc gcc tgc ctg ctt gct cag gga gaa 1183
 Trp Glu Val Val Glu Ala Thr Cys Ala Cys Leu Leu Ala Gln Gly Glu
 245 250 255

gag gcc gag aaa gaa cac tgc tcc aag tgc ctg gca gag cag atg atc 1231
 Glu Ala Glu Lys Glu His Cys Ser Lys Cys Leu Ala Glu Gln Met Ile
 260 265 270 275

ctg gag gaa ttt gga agg tgc tta tca cag att ctc cac act gag ttt 1279
 Leu Glu Glu Phe Gly Arg Cys Leu Ser Gln Ile Leu His Thr Glu Phe
 280 285 290

aaa tct aag gga ttg aaa atg gag tagagtataa agtgtgaatg catgttgatt 1333
 Lys Ser Lys Gly Leu Lys Met Glu
 295

ttgtcttagt ctagaaatct ctagttttaga aaggatgttt aggggaacat gaggctggct 1393

ctgcagcaac aaccaggctc ccctgcatcc ctgggccag ggagtttact cagagctctc 1453

tgaagatgtg gcaacccatg ccccttttct tgaggaggtg catggcctga gcattgtttg 1513

tctggcccag aggagagagc ttgggttccc atagtctctgg gagagtgtct gcagggcggc 1573

ggagggcaga gcaggcctg cggagagctc actctggtcg actcttctc tcagagaatg 1633

ttgctctgga ggctgctctg catgaaaacc ctaatggttt cttgtttggt tttcaaatta 1693

tttagaaata agttctccgg atgggctggt gtgataccac ttaaaatctc tagagaacta 1753

ctgaacacct aaagattttc tgtagcgtag atatttcccc agagacacgc gaactgtcag 1813

tctttcctaa ggccccggg agacgcaggc aatggggcct cgcaggccag gcttgcacca 1873

gcatgtcttg agttagagga cttaaaatta tccagtttct tctgtgtttc tacttgaatt 1933

gtggaaaagc tctattatcc aattaacttc tccataatta ttgttgtaat attattattg 1993

tttgtaaac atggttcaca taactagctt gtggaaacca gcaggtaaaa tgaattctta 2053

agttgacgct tttggttctg ttgtaaagca aagatgaata aaaatttcca atgtcgaaaa 2113

aaaaaaaaaa a

2134

<210> 4

<211> 295

<212> PRT

<213> Mus musculus

<400> 4

Met Val Ile Cys Gln Leu Lys Gly Gly Ala Gln Met Leu Cys Ile Asp
1 5 10 15

Asn Cys Gly Ala Arg Glu Leu Lys Ala Leu His Leu Leu Pro Gln Tyr
20 25 30

Asp Asp Gln Ser Ser Phe Pro Gln Ser Glu Leu Pro Lys Pro Met Thr
35 40 45

Thr Leu Val Gly Arg Leu Leu Pro Val Pro Ala Lys Leu Asn Leu Ile
50 55 60

Thr Gln Val Asp Asn Gly Ala Leu Pro Ser Ala Val Asn Gly Ala Ala
65 70 75 80

Phe Pro Ser Gly Pro Ala Leu Gln Gly Pro Pro Lys Ile Thr Leu Ser
85 90 95

Gly Tyr Cys Asp Cys Phe Ser Ser Gly Asp Phe Cys Asn Ser Cys Ser
100 105 110

Cys Asn Asn Leu Arg His Glu Leu Glu Arg Phe Lys Ala Ile Lys Ala
115 120 125

Cys Leu Asp Arg Asn Pro Glu Ala Phe Gln Pro Lys Met Gly Lys Gly
130 135 140

Arg Leu Gly Ala Ala Lys Leu Arg His Ser Lys Gly Cys Asn Cys Lys
145 150 155 160

Arg Ser Gly Cys Leu Lys Asn Tyr Cys Glu Cys Tyr Glu Ala Lys Ile
165 170 175

Met Cys Ser Ser Ile Cys Lys Cys Ile Ala Cys Lys Asn Tyr Glu Glu
180 185 190

Ser Pro Glu Arg Lys Met Leu Met Ser Thr Pro His Tyr Met Glu Pro
195 200 205

Gly Asp Phe Glu Ser Ser His Tyr Leu Ser Pro Ala Lys Phe Ser Gly
210 215 220

Pro Pro Lys Leu Arg Lys Asn Arg Gln Ala Phe Ser Cys Ile Ser Trp
225 230 235 240

Glu Val Val Glu Ala Thr Cys Ala Cys Leu Leu Ala Gln Gly Glu Glu
245 250 255

Ala Glu Gln Glu His Cys Ser Pro Ser Leu Ala Glu Gln Met Ile Leu
 260 265 270

Glu Glu Phe Gly Arg Cys Leu Ser Gln Ile Leu His Ile Glu Phe Lys
 275 280 285

Ser Lys Gly Leu Lys Ile Glu
 290 295

<210> 5

<211> 299

<212> PRT

<213> Homo sapiens

<400> 5

Met Val Ile Cys Gln Leu Lys Gly Gly Thr Gln Met Leu Cys Ile Asp
 1 5 10 15

Asn Ser Arg Thr Arg Glu Leu Lys Ala Leu His Leu Val Pro Gln Tyr
 20 25 30

Gln Asp Gln Asn Asn Tyr Leu Gln Ser Asp Val Pro Lys Pro Met Thr
 35 40 45

Ala Leu Val Gly Arg Phe Leu Pro Ala Ser Thr Lys Leu Asn Leu Ile
 50 55 60

Thr Gln Gln Leu Glu Gly Ala Leu Pro Ser Val Val Asn Gly Ser Ala
 65 70 75 80

Phe Pro Ser Gly Ser Thr Leu Pro Gly Pro Pro Lys Ile Thr Leu Ala
 85 90 95

Gly Tyr Cys Asp Cys Phe Ala Ser Gly Asp Phe Cys Asn Asn Cys Asn
 100 105 110

Cys Asn Asn Cys Cys Asn Asn Leu His His Asp Ile Glu Arg Phe Lys
 115 120 125

Ala Ile Lys Ala Cys Leu Gly Arg Asn Pro Glu Ala Phe Gln Pro Lys
 130 135 140

Ile Gly Lys Gly Gln Leu Gly Asn Val Lys Pro Gln His Asn Lys Gly
 145 150 155 160

Cys Asn Cys Arg Arg Ser Gly Cys Leu Lys Asn Tyr Cys Glu Cys Tyr
 165 170 175

Glu Ala Gln Ile Met Cys Ser Ser Ile Cys Lys Cys Ile Gly Cys Lys
 180 185 190

Asn Tyr Glu Glu Ser Pro Glu Arg Lys Thr Leu Met Ser Met Pro Asn
 195 200 205

Tyr Met Gln Thr Gly Gly Leu Glu Gly Ser His Tyr Leu Pro Pro Thr
 210 215 220

Lys Phe Ser Gly Leu Pro Arg Phe Ser His Asp Arg Arg Pro Ser Ser
 225 230 235 240

Cys Ile Ser Trp Glu Val Val Glu Ala Thr Cys Ala Cys Leu Leu Ala
 245 250 255

Gln Gly Glu Glu Ala Glu Lys Glu His Cys Ser Lys Cys Leu Ala Glu
 260 265 270

Gln Met Ile Leu Glu Glu Phe Gly Arg Cys Leu Ser Gln Ile Leu His
 275 280 285

Thr Glu Phe Lys Ser Lys Gly Leu Lys Met Glu
 290 295

<210> 6
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Primer

<400> 6
 ggagaatctg cgacaggcac

20

<210> 7
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Primer

<400> 7
 tccccagcca agttctcsgg

20

<210> 8
 <211> 21
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Primer

<400> 8
 ttcattgacc tcaactacat g

21

<210> 9
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 9

gtggcagtga tggcatggac

20

<210> 10

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 10

tatgggcgcc tcctttcagc tgacaaat

28

<210> 11

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 11

actgaggaag cagatggagc gctttgag

28

<210> 12

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 12

tgactgaggg aaactgctct ggtcat

26

<210> 13

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 13

aacctgatgg ctggcttgat

20

<210> 14

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 14

tttttcttta ctttccttgg

20

<210> 15

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 15

ccgaagaagg gctccaagaa

20

<210> 16

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 16

tctccactca agacaagcct

20

<210> 17

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 17

tgggcccagg gagtttactc a

21

<210> 18

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 18

tctcccagga ctatgggaac ccaa

24

<210> 19
 <211> 33
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Primer

<400> 19
 tcgaattcta tgggtgatttg tcagctgaaa gga

33

<210> 20
 <211> 32
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Primer

<400> 20
 gaattcgaat tcgcattccc ctttaccagc tt

32

<210> 21
 <211> 39
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Primer

<400> 21
 accgtcgact gcctaaggtc ctgagaactt ggctgggga

39

<210> 22
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic
 peptide

<400> 22
 Cys Leu Ser Gln Ile Leu His Ile Glu Phe Lys Ser Lys Gly Leu Lys
 1 5 10 15

Ile Glu

<210> 23
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<220>

<221> MOD_RES

<222> (2)

<223> Arbitrary amino acid

<220>

<221> MOD_RES

<222> (4)..(12)

<223> Arbitrary amino acid

<220>

<221> MOD_RES

<222> (14)

<223> Arbitrary amino acid

<400> 23

Cys Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Cys
 1 5 10 15

<210> 24

<211> 295

<212> PRT

<213> Mus sp.

<400> 24

Met Val Ile Cys Gln Leu Lys Gly Gly Ala Gln Met Leu Cys Ile Asp
 1 5 10 15

Asn Cys Gly Ala Arg Glu Leu Lys Ala Leu His Leu Leu Pro Gln Tyr
 20 25 30

Asp Asp Gln Ser Ser Phe Pro Gln Ser Glu Leu Pro Lys Pro Met Thr
 35 40 45

Thr Leu Val Gly Arg Leu Leu Pro Val Pro Ala Lys Leu Asn Leu Ile
 50 55 60

Thr Gln Val Asp Asn Gly Ala Leu Pro Ser Ala Val Asn Gly Ala Ala
 65 70 75 80

Phe Pro Ser Gly Pro Ala Leu Gln Gly Pro Pro Lys Ile Thr Leu Ser
 85 90 95

Gly Tyr Cys Asp Cys Phe Ser Ser Gly Asp Phe Cys Asn Ser Cys Ser
 100 105 110

Cys Asn Asn Leu Arg His Glu Leu Glu Arg Phe Lys Ala Ile Lys Ala
 115 120 125

Cys Leu Asp Arg Asn Pro Glu Ala Phe Gln Pro Lys Met Gly Lys Gly
 130 135 140

Arg Leu Gly Ala Ala Lys Leu Arg His Ser Lys Gly Cys Asn Cys Lys
 145 150 155 160
 Arg Ser Gly Cys Leu Lys Asn Tyr Cys Glu Cys Tyr Glu Ala Lys Ile
 165 170 175
 Met Cys Ser Ser Ile Cys Lys Cys Ile Ala Cys Lys Asn Tyr Glu Glu
 180 185 190
 Ser Pro Glu Arg Lys Met Leu Met Ser Thr Pro His Tyr Met Glu Pro
 195 200 205
 Gly Asp Phe Glu Ser Ser His Tyr Leu Ser Pro Ala Lys Phe Ser Gly
 210 215 220
 Pro Pro Lys Leu Arg Lys Asn Arg Gln Ala Phe Ser Cys Ile Ser Trp
 225 230 235 240
 Glu Val Val Glu Ala Thr Cys Ala Cys Leu Leu Ala Gln Gly Glu Glu
 245 250 255
 Ala Glu Gln Glu His Cys Ser Pro Ser Leu Ala Glu Gln Met Ile Leu
 260 265 270
 Glu Glu Phe Gly Arg Cys Leu Ser Gln Ile Leu His Ile Glu Phe Lys
 275 280 285
 Ser Lys Gly Leu Lys Ile Glu
 290 295

APPENDIX B

**Blast 2 Sequences results**

PubMed

Entrez

BLAST

OMIM

Taxonomy

Structure

BLAST 2 SEQUENCES RESULTS VERSION BLASTN 2.2.6 [Apr-09-2003]

Match: Mismatch: gap open: gap extension:
x_dropoff: expect: wordsize: Filter ☒

Sequence 1 gi 4581562 Homo sapiens tesmin mRNA, complete cds. Length 2134 *Sug. hora*

Sequence 2 gi 3206147 Sequence 1 from patent US 5700927 Length 4039 *ZON*

No significant similarity was found



Blast 2 Sequences results

[PubMed](#)[Entrez](#)[BLAST](#)[OMIM](#)[Taxonomy](#)[Structure](#)

BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.2.6 [Apr-09-2003]

Matrix **BLOSUM62** gap open: **11** gap extension: **1**

x_dropoff: **15** expect: **10.00** wordsize: **3** Filter ☒

Sequence gi metallothionein-like 5, testis-specific (tesmin); tesmin [Homo
1 4758742 sapiens] *Sugihara* Length 299

Sequence gi Sequence 2 from patent US 5700927 *ZON* Length 1141
2 3206311

No significant similarity was found.



NCBI Nucleotide

PubMed

Nucleotide

Protein

Genes

Structure

PMC

Taxonomy

OMIM

Book

Search for

Show:

1: I86429. Sequence 1 from p...[gi:3206147] (Nucleotide)

LOCUS I86429 4039 bp DNA linear PAT 10-JUN-1998
DEFINITION Sequence 1 from patent US 5700927.
ACCESSION I86429
VERSION I86429.1 GI:3206147
KEYWORDS
SOURCE Unknown.
ORGANISM Unknown.
REFERENCE 1 (bases 1 to 4039)
AUTHORS Zou, L. and Richardson, P.
TITLE Tbc1 gene and uses thereof
JOURNAL Patent: US 5700927-A 1 23-DEC-1997;
FEATURES
source Location/Qualifiers
1..4039
/organism="unknown"
/mol_type="unassigned DNA"
BASE COUNT 1042 a 1096 c 1060 g 841 t
ORIGIN

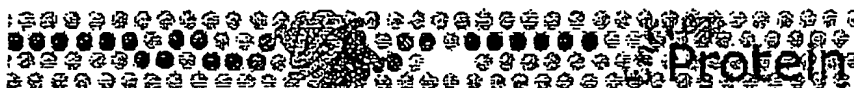
```
1 acctcggacc gaccgtctct tcaaccgcga agagaagcag agactgtgga gtggaccccc
61 accccagcag cgtggggcgg caagagcaca gccagctgag gaccatggac gccccgcccg
121 ccaggcccat cttctgggtg cccgggagca cctgcgcgoc ctagctcagc gcggagtctc
181 gagcgtgcca cctcactgga ttctgcoctc agaagcacgt gaaatagatc acttcattta
241 aagtaggaaa atagtagcac gtttttaccc gctc-ccaga cctctcccag gatggaggca
301 atcacattca cagcgaggaa gcatcggttt ccctaaccga gtctcogtgg acttcggctt
361 gcagctgggt ggggtctcctt acccgtgcac tctctacca ctatgcccac gctgcogtgg
421 gtggtagccg aggtacgaag actcagtgcc cagtgtctta aaaaggagcc taggacaaag
481 caagtccgcc tttgggtttc gccctccgga ctgcggtgcg agcctgacct ggagaaaagt
541 caaccatggg acccgtctat ctgttccage atctttgagt gcaagcctca gcgtgtgcac
601 aaactgattc acaacagtca cgaccgagc tactttgctt gcctcattaa ggaggatgct
661 gcgcacaggo agagcctctg ctatgtgttt aaagcagacg atcaaacaaa agtgcctgag
721 atcatcagct ccatccggca ggccgggaag attgcccgcg aggaagagct gcgttgcccc
781 tccgagttcg acgatacctt cgcacaaaag ttccaggtgc tcttctgtgg cggggtgact
841 gtggctcaca agaaggcccc acccgcactg attgacgagt gtatogagaa gttcaaacat
901 gtgagctgtg gtcgcagaac ggactgggaa gcgcccaccc ggcagccatc agcgccctggc
961 ccaggcccca tgcgcacaat cttctcacag cctggactgc gc-ctgctggc cttcaggaag
1021 gagttccagg acgctagcct ccgcagtagc acctttagct cc-cttgaca tgacatagag
1081 aaccacotca tcggtgggca caatgtgggt cagccacacg acatggagga gaacogaact
1141 atgctgttca cgattggccc atctgaagtt tactctatca gtcctgacac caaaaagatt
1201 gcaactggga aaaattttta ggagatatcc ttttgotctc agggcatcag acatgtggac
1261 cactttggat toatctgccg agagtgtctg ggtggcggca gtggcggtt tcattttgtc
1321 tgttacgtgt tccagtgcac aaatcaagct ctggttgacg agatcatgat gactctgaag
1381 caggctttca cggtagctgc ggtgcagcag acggctaagg caccagccca gctctgtgag
1441 ggctgcccct tgcaaggcct gcacaagctc tgogaaagga tagagggaat gaattcatct
1501 aaaaccaa atagaactcca gaagcaactg accacactga ccaa-cagga gcaggccacc
1561 atattcgagg aggttcagaa attgagacca agaaacagac agcgagagaa tgaattaatt
1621 attctttc tgaggtgctt atatgaagag aagcaaaaag agcacagcca cactggggcg
1681 ccaagcaga cactacaggt ggcagcagag aatattggga gtgacctgcc acccagtgct
```

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=nucleotide&list_uids=3206147&d..

1741 agcgggttca ggttagattc gctgaagaac agagcaaaga ggtccttaac agagtcccta
1801 gagagcattc tgtcccgagg taataaagcc agaggcctgc aggaocattc cgccagtgtg
1861 gatctggaca gctccacttc tagtactcta agtaacacca gcaaagagct gtccatgggt
1921 gacaaggagg ccttccccgt ctctgagacc tccctcaagc tccctggctc ctccagatgao
1981 ctgtccagtg actcagaggg ccacattgca gaagagtctg cctgtgtgtc accccagcag
2041 gcgttcagaa ggagagccaa caccctgagt cttttcccag tagagtgcc tgccctcca
2101 gaacctgccc agagtctcc aggggtctct caaaggaaac tcatgcggtt ccactccgtg
2161 agcacagaga cgcctcatga acgcaaggao tttgaatcca aagcaaacca cctgggtgac
2221 acagatggga ccccggtgaa gaccggggg cactcgtgga gacagcagat attccttoga
2281 gtggccactc cacagaaggg ttgtgactcc ccgagcagat atgaagatta ttccgagctg
2341 ggagagctcc ctccacgctc cctcttagaa ccgtgtgtgt aggcgggcc atttggccag
2401 tacagggaag aaagaggaag acgtcaogcg agcttcgaga gctgtggaag aaggccatct
2461 tgcagcagat cctgcctcgt caggatggag aaggagaate agaagctaca agcctotgaa
2521 aacgatttgc tgaacaaacg cctcaagctt gactatgaag aaatcactcc gtgtcttaa
2581 gaagtcacta cagtgtggga aaagatgctt agcactccag gaagatccaa aattaagttt
2641 gacatggaaa aagtgcactc agctgttggg caaggtgtgc cactcatca cccaggtgag
2701 atctggaaat ttctagctga gcagttccac cttaaacacc catttctag taaacagcag
2761 ccaaaggagc tgcctacaa agagctcctg aagaagctga cctcgcagca gcacggcatt
2821 ctcatcgacc tcgggcgaac ctttccaaca catccatact tctctgcca gcttgagca
2881 ggtcagctgt cactttacaa cattctgaag gcctactcgc ttctggacca ggaggttga
2941 tactgccaag gtctcagctt tgtggcaggg attttgcttc ttccatgag tgaggaagag
3001 gcgttcaaga tgctcaagtt cctgatgttt gacatggggc tgccgaaaca gtatcgcca
3061 gacatgatta ttttgagat ccagatgtac cagctgtcac ggctcctcca cgattaccac
3121 cgagacctct acaaccacct ggaagagcac gagactggcc cccctacgta ccggtctccc
3181 tgggtttctca ccgtgttgc ctcacagttc ccaactcggt ttgtagccag agtctttgat
3241 atgatcttcc ttcagggatc agaggtcata tttaaagtag ctttaagtct tttggggagc
3301 cataagccct tgattctaca gcatgagaac ctggaaacca tcgtggactt cataaagaac
3361 acaactccca acctgggctt ggtgcagatg gagaagaaca tcagtcaggt gtttgagatg
3421 gacatcgcca agcagctcca ggcctatgag gtccagatcc acgtcgtcca ggaggagctt
3481 attgagtcct cgcctctcag tgacaacca aagaatggaga aattggagaa aaccaacagc
3541 acgttgcgca aacagaacct tgacctctg gacagattgc aggtggcaaa tgctaggatc
3601 caaagccttg aagccaoggt agagaaactt cttaccagcg agagtaagct gaagcagcgt
3661 gcgctgaccc tggaggtgga gcgtcgccct gotgcagatg gtggaggagc tgccgaggca
3721 aagcgcccg cccagcactc cagagccaga ctgcacccag ctggagccca caggcgattg
3781 accgctgcca gaagagactg tgcaccatta acactgtcca agccttaate aagagagatg
3841 gaagtccagag gcagagaaga gagaacttct caggaggaa actggctgac cagcctgcag
3901 atccttttga gctcagaact tgggattgga ggacaaaagt ctccagagta ttgttgtttt
3961 tggttctaat ccgtccctt tccagtcctg gttgtgtgag ctttagatgg catggacatg
4021 aataaattac atttatggc

[Disclaimer](#) | [Write to the Help Desk](#)
NCBI | NLM | NIH

Rep 4 2005 10:24:24



PubMed Nucleotide Protein Genome Structure PMC Taxonomy OMIM Bio

Search for

Show:

1: AAC19746. Sequence 2 from p...[gi:3206311]

(protein)

BLink, Links

LOCUS I86593 1141 aa linear PAT 10-JUN-1998
 DEFINITION Sequence 2 from patent US 5700927.
 ACCESSION AAC19746
 VERSION AAC19746.1 GI:3206311
 DBSOURCE lccus I86593 accession AAC19746.1
 KEYWORDS
 SOURCE Unknown.
 ORGANISM Unknown.
 Unclassified.
 REFERENCE 1 (residues 1 to 1141)
 AUTHORS Zou, L. and Richardson, P.
 TITLE Tbc1 gene and uses thereof
 JOURNAL Patent: US 5700927-A 2 23-DEC-1997;
 FEATURES Location/Qualifiers
 source 1..1141
 /organism="unknown"

ORIGIN

```

1 mpmlpwvvaevrrlsggcakkeprtkqvr1 wvpsqglrcepdleksqpwd plicssifec
61 kqqrwhkllinhshdpsyfacklikedaahrqslcyvikadd qtkvpeilissircagkiarq
121 eelrcpsefddtfakkfevlfcgrvtvahk kappalideciekfnhvascrrtdweaptg
181 qpsapgprpmrkfsqpglrslafrkefqdaslrstfssfdndienhli gghnvvqptd
241 meenrtmlftigpsevylispdtkkialek nfkeisfcsq girhvdhfgf icrecsgggs
301 ggfhfvcyvftqctnealvdeimntlkqaftvaavqqataka paqlcegcpl qglhkiceri
361 egmnsskktkelqkhlttlt ngeqatifeevqklrprneqreneliisfl rclyeekqke
421 hshtgapkqtlqvaaenigsdlppsasrfrldslknrakr slteslesil srgnkarglq
481 dhsasvdlldststlnts kelsmgdkea fpvsetsfkl lgssddlssd seghiaeesa
541 llspqqafrrrantlshfpv ecpappespaq sspgvsqrkl mryhsvstet pherkdfesk
601 anhlgdtdgt pyktrrhswr qqiflrvatp qkacdpsry edyselgelp prsplepvce
661 dgpfgqyrkk rgrrhasfescgkrpccsrclvrmekenq klqasendll nkrlkldyee
721 itpclkevttvwekrlstpg rskikfdmek vhsavgggvr rhhrgeiwwkf laeqfhlkhp
781 fpskqgpkdvpkellkklt sqqhailidl grtfpthpyf saqlgagqls lynilkaysl
841 ldqevgyccgl sfvagilll hmseeeafkm lkflmfcmgl rkqyrpdmii lqlqmyqlsr
901 llhdylrdily nhleehetgptyaapwflt vfasqfplgf varvfdmifl qgsevifkva
961 lsllgshkpl ilqhenleti vdfikntlpn lgivqmekti sqvfamdiak qlqayeveyh
1021 vvqealiess plsdnqrmek lektnstlrk qnldilleqlq vanariqsle atveklitse
1081 sklkqraltl everrpaadg ggaseakrpa qhsrarlhpa gahrriitaar rdcapltlsk
1141 p
  
```

//

Disclaimer | Write to the Help Desk
 NCBI | NLM | NIH

Sep 4 1998 10:24:30

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=protein&list_uids=3206311&dopt...

SUBSTITUTE SHEET

testis library was screened. As a result, a clone having an approximately 1.7 kb length was obtained.

Moreover, 5'-RACE was conducted to determine the 5' end sequence. In 5'-RACE, three antisense primers specific to the Tesmin gene, namely, SP1 (SEQ ID NO: 10), SP2 (SEQ ID NO: 11), and SP3 (SEQ ID NO: 12), and mouse testis-derived 5'-Marathon RACE cDNA were used. 5'-RACE method was conducted following the ~~"Marathon-Ready™"~~ "Marathon-Ready™" cDNA kit (mouse testis)" (Clontech) protocol. The whole nucleotide sequence of Tesmin cDNA obtained is shown in SEQ ID NOs: 1 (2241 bp) and 2 (1861 bp). These two cDNAs are thought to be splicing variants arising from a difference in splicing at the point of transcription.

When the database was searched using these cDNA sequences, no sequence comprising a significant homology was found in the databank. These cDNAs encode the same protein comprising 295 amino acids (pI-7.64), and no significantly homologous proteins were found in the protein database as well.

Example 3: Cloning and sequencing of human Tesmin cDNA

Mouse Tesmin plasmid (a plasmid in which the Tesmin gene has been inserted into the pBluescript2 vector) was cleaved by SphI-SalI, and this 1.7 kb gene fragment was used as a probe to screen the cDNA library prepared by human testis mRNA. Hybridization was done using the "Rapid-hyb buffer" (Amersham LIFE SCIENCE) under the following conditions: (i) a prehybridization at 60°C for 30 min, (ii) addition of the labeled probe, and (iii) hybridization by incubating at 60°C for 2 hr. After that, washing is done three times within 2x SSC, 0.01% SDS for 20 min at room temperature, and next, three times within 1x SSC, 0.1% SDS, at 37°C for 20 min, followed by, two times within 1x SSC, 0.1% SDS, at 50°C for 20 min.

The nucleotide sequence of thus obtained human Tesmin cDNA is shown in SEQ ID NO: 3. Database search for the determined nucleotide sequence was done but there were no homologous sequences within the databank, similar to the mouse cDNA. The obtained human cDNA had four amino acids more than mouse Tesmin and encoded a

SUBSTITUTE SHEET

protein comprising 299 amino acids (pI-7.71). No significant homology was found in the protein database as well. However, as a result of amino acid sequence analysis by BLAST, the mouse and human Tesmins were found to be cysteine-rich proteins partially having the structure very similar to the metal-binding domain of the metallothionein family.

Metallothionein expression in the liver is induced by heavy metals, and metallothionein is known as a protein that neutralizes metallic poison. However, in the testis, the metallothionein gene is constantly expressed and is not induced by metals. Therefore, it was thought to play some vital roles other than metal binding in the testis. Recent findings showed that the estrogen receptor, which is a zinc-finger transcription factor and a receptor protein, and metallothionein conduct metal transfer *in vitro* (Cano-Gauci, D. and Sarkar, B. (1996) FEBS Lett 386 (1):1-4). Therefore, the metal-binding site of metallothionein is thought to play a vital role in the regulation of transcription factors. The "Cys-X-Cys-X-X-X-X-X-X-X-X-Cys-X-Cys (where X is an arbitrary amino acid)" sequence (SEQ ID NO: 23) having a cysteine structure in the amino acid sequence is thought to be vital for metal binding in the metallothionein family.

This cysteine structure (in mouse, from the 157th to the 171st positions, in human, from the 161st to the 175th positions) was conserved in Tesmin too. However, the metallothionein family members known up to now were relatively low-molecular comprising 60 to 70 amino acids, whereas Tesmin was comparatively longer (mouse: 295 amino acids, human: 299 amino acids). Domain search by PROSITE revealed that mouse Tesmin had a N-myristylation site and a casein kinase 2 phosphorylation site. Human Tesmin had a cAMP and cGMP-dependent kinase phosphorylation site, a protein kinase C phosphorylation site, a N-myristylation site, and a N-glycosylation site. Other than those, a cAMP and cGMP-dependent protein kinase phosphorylation site and a protein kinase phosphorylation site were also present.

Domain search by BLOCKS revealed sites common to mouse and human Tesmins. Namely, "high potential iron-sulfate protein"